REMARKS / ARGUMENTS

Claims 1-8 are currently pending in the application. Claims 1-8 have been rejected. Claims 1 and 4 have been amended. No new matter has been added by way of this amendment, nor has the scope of this claim been narrowed. Support for amended claims 1 and 4 can be found in the specification, *e.g.*, at page 12, lines 8-12, and original claims 1 and 4.

Further, new claims 9-20 are added for consideration. New claims 9-20 are fully supported by the application as filed and thus present no new matter. Specific support for new claims 9-12 may be found, for example, in the specification at page 33, line 14. Specific support for new claims 13-20 may be found, for example, in the specification at page 10, lines 1-10.

Following entry of this amendment, claims 1-20 will be pending in this application. Applicants respectfully request reconsideration of pending claims 1-20.

The Examiner has indicated that "Claims 4-8 are allowed" (Office Action, page 10) However, claims 4-8 have been rejected by the Examiner under 35 U.S.C. § 112, second paragraph (Office Action, pages 2, 5 and 6). Clarification is respectfully requested.

I. Rejection Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 1-8 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite (Office Action, pages 2 and 5).

For completeness, each ground of rejection will be addressed separately below.

A, Claims 1 and 4 - "Capture Antigen"

The Examiner opines that claims 1 and 4 are unclear as to where the "capture antigen" is located with regard to the rare cell (Office Action, page 2).

Applicants respectfully traverse this ground of rejection.

As discussed in Applicants' prior Response dated June 29, 2005, the specification describes the meaning of "capture antigen":

> A sample is then prepared by mixing the biological specimen with magnetic particles which are coupled to a bispecific ligand specifically reactive with an antigen on the rare cell that is different from or not found on blood cells (referred to herein as a "capture antigen"), so that other sample components may be substantially removed.

(Specification, page 3, lines 21-25).

> Capture antigens may be any cell surface antigen that is differentially expressed on the target cells. Preferably, capture antigens are cell surface receptors that are expressed exclusively on the target cells, or that are over expressed on the target cells relative to other cells in circulation. Magnetic particles are provided that have attached an antibody composition specific for such capture antigen.

(*Id.* at page 12, lines 8-12). Non-limiting examples of capture antigens are provided in the specification, *e.g.*, at page 33, lines 12-25.

However, solely in an effort to advance prosecution of this application, Applicants have amended claims 1 and 4 to recite that the antibody composition is specific for a cell surface antigen of the rare cell type. No new matter has been added by way of this amendment, and support can be found in the specification, *e.g.* at page 12, lines 8-12.

B. Claim 4 - Last Step

The Examiner opines that claim 4 is indefinite because the last step is allegedly inconsistent with the preamble of the claim (Office Action, page 2). Claim 4 has been amended herein to delete "biomarkers" and recite instead "protein-protein complexes." No new matter has been added by way of this amendment.

C. Claim 1 - Step 3

The Examiner opines that claim 1 is indefinite because step 3 recites "one or more" biomarkers, whereas step 4 recites "a plurality" of biomarkers (Office Action, page 5). Claim 1 has been amended to combine the language of the second and third "steps" to more

precisely claim the invention. As such, the "a plurality of" biomarkers language has been deleted. No new matter has been added by way of this amendment.

D. Claim 1 - More than one biomarker detected.

The Examiner opines that claims 1 and 4 are "confusing":

> [W]hile there is only one cell type isolated, there are more than one biomarkers being detected. Usually if the cell type is a cancer cell, then there is one cancer biomarker on the cell. Thus, there would be only one kind of tag for such one type of cell or biomarker...However, claim 1 has a plurality of tags and then separating the released tags [*sic*]. If there is only one kind of tag being used for one kind of biomarker, then what other cleaved tags are there to be separated at the end before identifying the cleaved tags

(Office Action, pages 5-6).

Respectfully, the Examiner seems to be confusing the concepts of (a) antibody and capture antigen (isolation or rare cell type), and (b) binding compound and biomarker (detection and identification of biomarkers).

The invention provides methods for detecting one or more biomarkers of a rare cell type (*e.g.*, a cancer cell). In certain embodiments, the methods provide that an antibody composition that binds to a rare cell type cell surface antigen is used to isolate a subpopulation of cells comprising the rare cell type. As those skilled in the art are aware, *e.g.*, one cancer cell type may have more than one cell surface antigen that may be used for isolation. As such, claim 1 provides that "one or more antibody compositions" may be used.

The methods also provide that a binding compound releasably attached to a molecular tag is combined with the rare cell type, such that the binding compound forms a complex with a given biomarker of the rare cell type. One skilled in the art would understand that the rare cell type (*e.g.*, a cancer cell) may have numerous biomarkers (see, specification at page 1, lines 19-21). Thus, in some embodiments, the invention provides for the detection with a given binding compound of only one biomarker of a rare cell type. In other embodiments, the invention provides for the detection of more than one biomarker of a rare cell type with more than one binding compound.

Each binding compound used to detect a biomarker of a rare cell type comprises a releasably attached molecular tag, wherein each molecular tag can have distinct peak in a separation profile. For example, when more than one biomarker is being detected in a given rare cell type subpopulation, more than one molecular tag is released and separated. As such, each different releasable tag in a separation profile will correlate with the presence of a given biomarker in the sample.

Accordingly, Applicants respectfully submit that claim 1 is clear and unambiguous, and one skilled in the art would not deem the claimed methods "confusing."

E. Claim 4 - More than one protein complexes detected.

The Examiner also asserts that claim 4 is "confusing" (Office Action, pages 5-6). The Examiner opines that claim 4 is "unclear of whether the first protein and the second protein are the same as antibody composition and capture antigen, respectively" (Office Action, page 6). However, the Examiner again seems to be confusing the concepts of (a) antibody and capture antigen (isolation or rare cell type), and (b) first and second binding compounds and first protein-second protein complexes (detection and identification of protein-protein complexes).

Claim 4 provides a method of detecting one or more protein-protein complexes of a rare cell type (*e.g.*, cancer cell). Similar to claim 1 discussed above, the method of claim 4 provides that an antibody composition that binds to a rare cell type cell surface antigen is used to isolate a subpopulation of cells comprising the rare cell type. As those skilled in the art are aware, *e.g.*, one cancer cell type may have more than one cell surface antigen that may be used for isolation. As such, claim 1 provides that "one or more antibody compositions" may be used.

The methods also provide that a first binding compound with a molecular tag releasably attached thereto and second binding compound (conjugated to a cleaving-inducing moiety having an effective proximity) is combined with the rare cell type, such that the first binding compound binds to a first protein in a protein-protein complex of the rare cell type and the second binding compound binds to a second protein in the protein-protein complex of the rare cell type. One skilled in the art would understand that the rare cell type (*e.g.*, a

cancer cell) may have numerous, different protein-protein complexes. Thus, in some embodiments, the invention provides for the detection of only one protein-protein complex of a rare cell type with one "set" of binding compounds (*i.e.,* a first binding compound and a second binding compound that bind to a first and second protein in a protein-protein complex, respectively). In other embodiments, the invention provides for the detection of more than one protein-protein complex of a rare cell type with more than one "set" of binding compounds.

Each first binding compound used to detect a first protein in a protein-protein complex of a rare cell type comprises a releasably attached molecular tag, wherein each molecular tag can have distinct peak in a separation profile. For example, when more than one protein-protein complex is being detected in a given rare cell type subpopulation, more than one molecular tag is released and separated. As such, each different releasable tag in a separation profile will correlate with the presence of a particular protein-protein complex in the sample.

Accordingly, Applicants respectfully submit that claim 4 is clear and unambiguous, and one skilled in the art would not deem the claimed methods "confusing."

Thus, for at least the reasons given in sections (A) - (E) above, Applicants submit that claims 1-8 are clear and unambiguous and satisfy all the requirements of 35 U.S.C. § 112, second paragraph. Accordingly, reconsideration and withdrawal of this ground of rejection is respectfully requested.

II. 35 U.S.C. § 112, First Paragraph.

The Examiner has rejected claims 1-3 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement (Office Action, pages 3-5).

Applicants respectfully traverse this ground of rejection.

The Examiner asserts that "the predictability is low" due to the "lack of a separation step of the bound from the unbound before cleaving the tags for analysis" and that the

specification "fails to provide guidance on how meaningful results could be obtained without separating the unbound from the bound tags before cleaving the tags for detection"(Office Action, page 4).

Contrary to the Examiner's assertions, the specification explains that a wash step is not required in all instances:

> After stable complexes are formed, between the biomarkers and the binding compounds, the molecular tags are released from the binding compounds forming stable complexes. In some embodiments, binding compounds failing to form stable complexes are removed, *e.g.,* in a wash step, after which molecular tags are release from the binding compounds forming the stable complexes. In such embodiments (*i.e.,* heterogeneous formats), as explained below, a wide range of cleavable linkages are available. In other embodiments, no wash step is preformed because a cleaving agent is employed that acts locally to a complex. After cleavage, the molecular tags are then separated and detected.

(Specification, page 12, lines 23-28; emphasis added).

> Biomarkers may be detected in assays having homogeneous formats or non-homogeneous, *i.e.,* heterogeneous formats. In a homogeneous format, no step is required to separate binding compounds specifically bound to target complexes from unbound binding compounds. In a preferred embodiment, homogeneous formats employ reagent pairs comprising (i) one or more binding compounds with releasable molecular tags, and (ii) at least one cleaving probe that is capable of generating an active species that reacts with and releases molecular tags within an effective proximity of the cleaving probe.

(*Id.* at page 19, lines 5-11; emphasis added).

Moreover, the specification provides extensive guidance with respect to the choice of cleaving agents, molecular tags, cleavable linkages and other components for homogeneous (as well as heterogeneous) assay formats (see, *e.g., Id.* at page 20, lines 8-11).

The specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski* , 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). Further, the number and variety of examples is irrelevant if the disclosure is "enabling" and

sets forth the "best mode contemplated." *In re Borkowski* et al. (CCPA 1970) 442 F2d 904, 164 USPQ 642.

Here, the Applicants have provided an assay format that does not require a wash step, as well as a number of cleaving agents, molecular tags, cleavable linkages and other components suitable for use in this assay formats. Therefore, one skilled in the art, given the extensive guidance provided in the specification, in conjunction with the knowledge and skill in the art at the time of filing, would be able practice the method of claim 1 without undue experimentation.

Thus, Applicants submit that claims 1-8 are fully enabled and comply with all the requirements of 35 U.S.C. 112, first paragraph. Accordingly, Applicants respectfully request that this ground of rejection be reconsidered and withdrawn.

III. Rejection Under 35 U.S.C. § 103(a)

A. Claim 1

Claim 1 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Terstappen *et al.* (U.S. Patent No. 6,365,362) ("Terstappen") in view of Ness *et al.* (U.S. Patent No. 6,815,212) ("Ness") (Office Action, pages 6-9).

Applicants respectfully traverse this ground of rejection.

The Examiner cites Terstappen as teaching a method of magnetically isolating a rare cell type from a mixed population of cells, the method comprising the steps of mixing colloid metal particles conjugated with a monoclonal antibody reactive with a rare cell determinant different than those found on blood cells, applying a magnetic field to isolate the bound complex from the unbound magnetic particle-antibody conjugates, adding a second set of monoclonal antibodies that are labeled with reporter molecules to the rare cell type, and detecting the bound portion (*Id.* at pages 6-7).

However, as appreciated by the Examiner, Terstappen fails to disclose or suggest molecular tags releasably attached to a binding compound, wherein the molecular tags have

distinct separation characteristics that form distinct peaks in a separation profile upon separation. Terstappen also fails to teach or suggest releasing the molecular tags of each binding compound, and separating and identifying the released molecular tags to determine the one or more biomarkers in the sample.

To supply the deficiencies of Terstappen, the Examiner cites Ness as teaching an assay method comprising combining a set of first tagged members with a biological sample containing a second member of a ligand pair to permit binding or formation of a complex between the first and second members, separating the bound first and second members from unbound members, cleaving the tag from the tagged first member, and detecting the tag by spectroscopic methods (*Id.* at pages 7-8).

However, Ness does not disclose or suggest a method of detecting one or more biomarkers of a rare cell type in a sample, comprising (1) immunomagnetically isolating a subpopulation of cells containing a rare cell type, (2) combining the rare cell population with a binding compound (releasably attached to a molecular tag) that forms a complex with a biomarker, and (3) releasing, separating and identifying the molecular tag(s) to determine the one or more biomarkers in the sample.

The Examiner opines that it would have been obvious to one skilled in the art to cleave and analyze the tags as taught by Ness as a detection step to detect biomarkers of cells isolated according to the method of Terstappen (*Id.* at page 8).

However, it is not sufficient that the prior art *can be* modified to produce the claimed invention: the modification is non-obvious unless the prior art suggests the desirability thereof. *In re Laskowski*, 10 USPQ 2d 1397 (Fed. Cir. 1989). Further, the invention as a whole must be considered when determining obviousness, rather than the obviousness of any substitution or modification. *Hybritech v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986).

Respectfully, the Examiner's assertion that modifications of the prior art to meet the claimed invention would have been obvious to one of ordinary skill in the art at the time the claimed invention was made because either the references relied upon teach that all aspects of the claimed invention, or were individually known in the art, is not sufficient to establish a

prima facie case of obviousness without some objective reason to combine the teachings of the references. See, *e.g., Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993). See also *Al-Site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999) (The level of skill in the art cannot be relied upon to provide the suggestion to combine references.); *see also* M.P.E.P. § 2143.01. Moreover, "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine,* 837 F.2d 1071 (Fed. Cir. 1988) (emphasis added).

Applicants submit that there is no motivation to combine the cited references, and the desirability of such a modification of the prior art is completely lacking. Terstappen claims to disclose "a highly sensitive assay...to detect, enumerate and characterize carcinoma cells in the blood" (Terstappen, Abstract). Terstappen also claims that "[t]he present invention provides a rapid and efficient screening method for the characterization of not only tumor cells, but also rare cells, or other biological entities from biological samples...The method described herein combines elements of immunomagnetic enrichment with multiparameter flow cytometric, microscopic and immunocytochemical analysis in a unique way...The sensitive nature of the assay facilitates the detection of residual disease, thus making it possible to monitor for cancer recurrence." (Col. 7, line 57 - Col. 8, line10).

Thus, because Terstappen touts the "highly sensitive assay" to characterize "rare cells," one skilled in the art would have no motivation to further combine the cleavable tags of Ness with immunomagnetic separation methods of Terstappen into a single biomarker detection assay. As such, Applicants respectfully submit that the Examiner has failed to establish *prima facie* of claim 1 over the cited references.

Thus, for at least these reasons, Applicants submit that claim 1 is non-obvious over Terstappen either alone or in combination with Ness. Accordingly, reconsideration and withdrawal of this ground of rejection is respectfully requested.

B. Claim 2

Claim 2 has also been rejected under 35 U.S.C. § 103(a) as being unpatentable over Terstappen in view of Ness, and further in view of Wels *et al.* (U.S. Patent No. 5,571,894) ("Wels") (Office Action, page 9).

Applicants respectfully traverse this ground of rejection.

Both Terstappen and Ness are discussed above. The Examiner cites Wels as discussing that growth factors and their receptors are involved in the regulation of cell proliferation and play a role in tumor growth (*Id.*). The Examiner also cites Wels as disclosing that "the c-erbB2 protein has potential both as a diagnostic marker and as a target for cancer therapy (*Id.*). The Examiner opines that:

> [s]ince Terstappen teaches using a kinase receptor as a receptor on [*sic*] cell for detecting cancer cells and Ness teaches a method of detecting cancerous cells, it would have been obvious to one of ordinary skills [*sic*] in the art to use antibodies that bind to erbB receptor of the tyrosine kinase receptor family as taught in Wels to detect cancer cells according to the combined method of Terstappen and Ness because cancer cells have erbB or tyrosine kinase receptor on their membrane.

(*Id.*).

However, even assuming *arguendo* that it was somehow obvious to use the erbB receptor as a surface antigen for immunomagnetically separating the rare cell type according to Terstappen, Wels does nothing to supply the deficiency of Ness (discussed above). That is, nowhere does Wels disclose or suggest a method of detecting one or more biomarkers of a rare cell type in a sample, comprising (1) immunomagnetically isolating a subpopulation of cells containing a rare cell type, (2) combining the rare cell population with a binding compound (releasably attached to a molecular tag) that forms a complex with a biomarker, and (3) releasing, separating and identifying the molecular tag(s) to determine the one or more biomarkers in the sample. Wels also does not provide any motivation to use the tagged members of Ness in the assay of Terstappen. As such, Applicant respectfully submit hat the Examiner cannot establish *prima facie* obviousness of claim 2 over the cited references.

Thus, for at least these reasons, Applicants submit that claim 2 is non-obvious over Terstappen and/or Ness, either alone or in combination with Wels. Accordingly, reconsideration and withdrawal of this ground of rejection is respectfully requested.

IV. Conclusion

In view of the foregoing remarks, Applicants respectfully submit that this application is now in condition for allowance. If a telephone interview would advance prosecution of the application, the Examiner is invited to call the undersigned at the number listed below.

A Petition for a one (1) month Extension of Time under 37 C.F.R. § 1.136(a) is filed concurrently herewith (in duplicate), which extends the response period from January 19, 2006 to February 19, 2006. Because February 19, 2006 falls on a Sunday, and Monday, February 20, 2006 is a Federal holiday within the District of Columbia (President's Day), the response period is extended to the next succeeding business day, Tuesday, February 21, 2006, pursuant to 37 C.F.R. § 1.7. The Petition further authorizes the PTO to charge the one month extension fee of $65 to our Deposit Account No. 50-3013, which reflects Applicant's Small Entity Status.

No additional fees are believed due in connection with this Amendment. However, if there are any other fees due, please charge them to Deposit Account 50-3013. If a request for extension of time and fee are required under 37 C.F.R. § 1.136 that have not been accounted for, such an extension is requested and the fee should be charged to our Deposit Account. Also, please charge any fees underpaid or credit any fees overpaid to the same Deposit Account.

Respectfully submitted,

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